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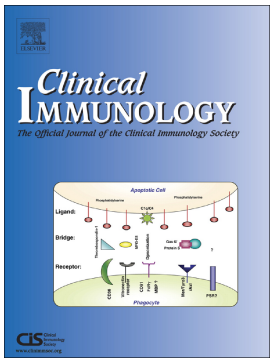

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CLEAN VERSION

High frequency of neutralizing antibodies to type I Interferon in HIV-1 patients hospitalized for COVID-19

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Abstract

The presence of anti-IFN neutralizing antibodies (NAB) has been reported in critically ill COVID-19 patients. We found that 87.5 % (7/8) of HIV-1 patients co-infected with SARS-CoV-2 had serum anti-IFN-I NAB against IFN-α subtypes, IFN-β and/or IFN-ω. Anti-IFN-I NAB were also detected in oropharyngeal samples. Patients with NAB were males, and those with high serum anti-IFN-α/ω NAB titer had severe illness and exhibited reduction in the expression of IFN-stimulated genes. Thus, high titer of anti-IFN-α/ω NAB may contribute to the greater severity of COVID-19 in HIV-1 infected patients.

Key words: HIV-1, SARS-CoV-2, neutralizing antibodies, Interferon, ISGs, HIV-1 and SARS-CoV-2 co-infection

To the editor:

How HIV-1 infection affects risk of severe COVID-19 outcome is poorly investigated. Evidence from different studies does not support a higher risk of SARS-CoV-2 infection in HIV-1 infected patients [1]; however, it has been reported that COVID-19 has a negative impact on HIV-1 individuals, especially in the presence of comorbidities that increase the risk of serious illness [2]. Moreover, there are multiple immunological profiles of HIV-1 infected individuals, and the impact of SARS-CoV-2 infection can vary for each patient [2]. A dysregulation of innate immunity has been observed in both HIV-1 infected individuals and severe COVID-19 patients. In particular, type I Interferon (IFN-I) signalling has been reported to exert a dichotomous role in the pathogenesis of acute *vs*. chronic HIV-1 infection [3]. Additionally, severe COVID-19 is characterized by a delayed or suppressed IFN-I response, in part due to evasive strategies employed by SARS-CoV-2 [4], as well as IFN genetic defects [5] and anti-IFN neutralizing antibodies (NAB) [6, 7]. Increasing evidence showed a dominant role of cell-mediated immunity in the clearance of SARS-CoV-2 in HIV-1 infected patients [8], while little is known about the impact of co-infection with SARS-CoV-2 and HIV-1 on antiviral innate immune responses.

We evaluated the presence of anti-IFN-I NAB in 8 HIV-1 positive individuals co-infected with SARS-CoV-2. During March 2020 to April 2021, blood samples were collected at the time of hospital admission for COVID from 6/8 HIV-1 patients seen at the Policlinic Umberto I hospital in Rome, Italy. For 2/8 patients, blood was collected at the time they first tested positive for SARS-CoV-2. No other common respiratory viruses [Respiratory syncytial virus A and B, Influenza A virus, Rhinovirus, and low pathogenetic human coronaviruses (HCoVs OC43, 229E, NL-63, and HUK1), and Metapneumovirus] were detected in the nasopharyngeal swabs of these patients [supplementary file 1 (S1)]. Paired nasopharyngeal swabs and serum samples collected at the time of hospitalization were available for 3 of the 8 patients. The study was approved by the ethics committee of the Policlinic Umberto I Hospital, and informed consent was obtained from participants. NAB to IFN-α2 subtype (Intron®; Schering-Plough), multiple IFN-α subtypes

contained in the natural IFN preparation (IFN-αn1, Wellferon®, Glaxo Wellcome, London, UK), IFN-β (Rebif, Serono, Geneva, Switzerland) and IFN-ω (PBL Interferon Source, Piscataway, USA) were measured using a bioassay based on IFN-induced inhibition of the cytopathic effect caused by encephalomyocarditis virus on human lung carcinoma epithelial cells (A549) (S1) [7].

Anti-IFN-I NAB against one or more IFN-I preparations were detected in serum samples from 7 of 8 (87.5%) of the HIV-1 patients (Table 1). The range of NAB levels against IFN-I was broad [10-530000 tenfold reduction unit (TRU/ml), Table 1)]. The frequency of patients with NAB against IFN-ω (5/8, 62.5%) was comparable to that observed for IFN-α2 (3/8, 37.5 %), IFN-αn1 (3/8, 37.5 %) and IFN-β (3/8, 37.5 %) (p=0.61 using Fisher exact test). Among anti-IFN-ω positive patients, two patients had anti-IFN-α NAB and another two patients had anti-IFN-β NAB. One patient had NAB exclusively to IFN-ω. One patient each had NAB to IFN-α or IFN-β in the absence of NAB to IFN-ω. No patient had NAB to both IFN-α and IFN-β (Table 1). The only patient with no detectable anti-IFN-I NAB was a female; all the male patients had detectable anti-IFN-I NAB. Two NAB positive patients died: one of whom (pt No. 4) showed a fatal outcome related to COVID-19 while the other one (pt No. 1) had a cerebral non-Hodgkin's lymphoma (Table 1). The following comorbidities were observed: hypertension (pt No. 7 and pt No. 8), hypercholesterolemia (pt No.7), diabetes (pt No. 8). Six out of eight (75%) NAB positive patients were hospitalized for a median of 52 days (range 21-110 days, Table 1). SARS-CoV-2/HIV-1 coinfected patients with the highest NAB titer (≥2100 TRU/ml, pt No. 2, 3, 4) had values of a COVID-19 severity Index [9] considered critical (8-11). Levels of laboratory biomarkers associated with major risks for severe COVID-19 [lactate dehydrogenases (LDH), CRP, fibrinogen and D-Dimer] [7] were high in all hospitalized patients, and further increased in those with higher NAB titers (Table 1). Levels of CD4 T cells were lower than 500 cells/μl in those patients (range CD4 T cell values: 86-304 cells/μl, patients No. 2, 3 and 4) with elevated NAB titer against IFN-I. No patients included in this study were previously treated with IFNα/β preparations or received COVID-19 vaccines before testing positive for SARS-CoV-2.

Previous studies have reported that 10% to 15% of patients with severe COVID-19 exhibit anti-IFN-I NAB [6, 7]. We showed that the proportion of HIV-1 and SARS-CoV-2 co-infected patients with NAB to IFN-I is much higher (75%). NAB against IFN-α are uncommonly detected in HIV-1 infected patients, except in those receiving IFN-α preparation with the aim of inducing anti-IFN-α antibodies to counteract IFN-α overproduction [10]. Thus, although none of our patients had been previously treated with IFN-α therapy, 3/8 patients had NAB against IFN-α2 and produced NAB against IFN-αn1 (Table 1), suggesting that those patients might have developed a broad spectrum of NAB with specificity against different IFN-α subtypes.

Because anti-IFN-I NAB have recently been detected in respiratory samples of SARS-CoV-2 positive patients [7], we measured NAB in respiratory samples from 3 SARS-CoV-2 and HIV-1 co-infected patients (Table 1). NAB against IFN-ω were detected in two oropharyngeal swab samples (Table 1); anti-IFN-α NAB (107 TRU/ml) were detected in one of these samples. No anti-IFN-β NAB were detected in oropharyngeal samples (Table 1).

High titres of serum NAB have been associated with reduction and/or abrogation of the endogenous induced IFN response in COVID-19 patients [7]. Therefore, we performed gene expression analysis of IFN stimulated genes (ISGs) that have been reported to be involved in immunopathogenesis of HIV-1 or are considered important antiretroviral restriction factors, such as ISG15 [11], APOBEC3G and APOBEC3F [12]. We compared mRNA levels in PBMCs from SARS-CoV-2 and HIV-1 co-infected patients positive for anti-IFN-I NAB (n=7), with levels in gender and age matched HIV-1 infected individuals (n=16) without SARS-CoV-2 infection and healthy donors (n=16, table 1). None of the healthy controls and HIV-1 mono-infected patients had detectable NAB in serum samples. The mRNAs levels of ISGs were measured in PBMCs by quantitative RT/real time PCR assays using LightCycler480 instrument (Roche, Basel, Switzerland) as previously reported (S1). Primers and probes for APOBEC3G (Hs.PT.58.27074917) and APOBEC3F (Hs.PT.58.2507020) were purchased from Integrated DNA Technologies. The following primers and probe were used for ISG15: ISG15 Forward 5′-TGGCGGGCAACGAATT-

3′, ISG15 Reverse 5′-TGATCTGCGCCTTCA-3′; ISG15 Probe 5′-6FAM-TGAGCAGCTCCATGTC-TAM-3′ [7]. Transcript levels of APOBEC3G and APOBEC3F were strongly reduced ($p<0.001$ for both genes using Mann Whitney test) in anti-IFN-I NAB positive co-infected patients [supplementary file 2 (S2)] [13]. A trend toward lower expression of ISG15 in NAB positive patients was observed compared to HIV-1 patients uninfected with SARS-CoV-2 ($p=0.50$) and healthy individuals ($p=0.77$) (S2). Moreover, we found an inverse correlation between ISG15 mRNA expression and the titer of NAB against IFN-α2 ($p=0.030$, Spearman rho=-0.544) and the natural IFN-α preparation ($p=0.041$, Spearman rho=-0.516) respectively. These results are consistent with those of our previous investigation, in which we reported decreased levels of ISGs in COVID-19 patients who had anti-IFN-α/ω NAB [7]. By contrast, no significant correlation was observed between NAB titer and APOBECs transcript levels, despite their mRNA levels were highly reduced in the presence of NAB (S2). The reason for these results remains unclear. Remarkably, SARS-CoV-2 has been shown to utilize the APOBEC-mediated mutations for fitness and evolution [14]; on the other hand, APOBEC levels were found to be downregulated in severe COVID-19 [13], highlighting the complexity of the phenomenon analyzed.

Our findings demonstrated for the first time a high rate of a broad spectrum of NAB with specificity against IFN-α subtypes, IFN-b, and IFN-ω in SARS-CoV-2 and HIV-1 co-infected patients. It is unknown whether the presence of anti-IFN-I NAB reflects pre-existing autoimmunity contributing to severe disease in some patients or if the appearance of NAB is in response to SARS-CoV-2-induced increase of IFN.

Detection of anti-IFN-I NAB might have value as a prognostic indicator for severe COVID-19 disease in HIV-1 infected patients. The presence of a high level of serum NAB against IFN-α and IFN-ω was associated with severe illness, although the range of NAB levels against these IFNs was very broad. Further studies with larger number of SARS-CoV-2 and HIV-1 co-infected patients, across the spectrum of SARS-CoV-2 associated disease, are needed to better characterize the clinical and biological significance of NAB in HIV-1 patients.

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Table 1. Neutralizing antibodies (NAB) to IFN-I in SARS-CoV-2 and HIV-1 co-infected patients.

Item	Patient No. 1	Patient No. 2	Patient No. 3	Patient No. 4	Patient No. 5	Patient No. 6	Patient No. 7	Patient No. 8	SARS-CoV-2 and HIV-1 co-infected patients (n=8)	HIV-1 positive patients without SARS-CoV-2 infection (n=16)	Healthy donors (n=16)
Gender	male	male	male	male	female	male	male	male	male/female: 7/1	male/female: 14/2	male/female: 14/2
Age (years)	42	78	57	50	47	51	53	80	54 (42-80)	56 (40-82)	57 (41-82)
HIV-1 RNA (copies/ml)[b]	<37	<37	<37	<37	<37	<37	<37	<37	<37	<37	NA
Years from HIV-1 diagnosis	21	13	8	18	15	13	17	3	14 (3-21)	15 (8-25)	NA
Years on ART	21	13	8	13	14	13	10	3	13 (3-21)	12 (6-23)	NA
Anti-HIV-1 drug class	Prezista	Descovy, Isentress	Descovy, Isentress	Descovy, Isentress	Descovy, Isentress	Descovy, Isentress	Biktarvy	Delstrigo	-	Emtricitabine n=6 (37.5%), Isentress n=3 (18.75%), Kaletra n=1 (6.25%), Kivexa n=3 (18.75%), Nevirapine n=5 (31.25%), Prezista n=3 (18.75%), Ritonavir-	NA

											Saquinavir n=6 (37.5%), Truvada n=1 (6.25%)	
CD4 T cell count (cells/µl)		>500	304	304	86	>500	>500	>500	>500	>500 (86->500)	>500 (210-1053)	NA
Hospitalization (days)		110	44	60	60	0	0	21	21	52 (21-110)	NA	NA
Biochemical parameters	LDH (UI/l)	198	551	201	333	NA	NA	234	280	257 (198-551)	NA	NA
	CRP (mg/dl)	25.47	15.52	13.46	1.66	NA	NA	2.90	0.23	8.18 (0.23-25.47)	NA	NA
	Fibrinogen (mg/dl)	401	591	555	532	NA	NA	555	412	543.5 (401-591)	NA	NA
	D-dimer (µg/l)	1918	2997	501	33933	NA	NA	238	240	1209.5 (238-33933)	NA	NA
COVID-19 Severity Index+		3	11	9	8	1	1	5	4	4.5 (1-11)	NA	NA
COVID-19 therapy*		Decadron, Velklury, Heparin	Decadron, Velklury, Heparin	Decadron, Velklury, Heparin	Decadron, Velklury, Heparin	Bamlanivimab Etesevimab, Heparin	Bamlanivimab Etesevimab, Heparin	Decadron, Velklury, Heparin	Decadron, Velklury, Heparin	-	NA	NA
Outcome of COVID-19		Dead	Survival	Survival	Dead	Survival	Survival	Survival	Survival	-	NA	NA
NAB status**												
IFN-α2 (TRU/ml)^	Serum	**53**	**530000**	<10	**5689**	<10	<10	<10	<10	3/8 (53-530000)	0/16 (<10)	0/16 (<10)
	Oropharyngeal swab	<10	**107**	<10	NA	NA	NA	NA	NA	1/8 (107)	NA	NA
IFN-	Serum	**13**	**1365**	<10	**8960**	<10	<10	<10	<10	3/8	0/16	0/16

αn1 (TRU/ ml)			**00**							(13-13650 0)	(<10)	(<10)
	Oropharyngeal swab	<10	**106**	<10	NA	NA	NA	NA	NA	1/3 (106)	NA	NA
IFN-β (TRU/ ml)	Serum	<10	<10	<10	<10	<10	**10**	**13**	**26**	3/8 (10-26)	0/16 (<10)	0/16 (<10)
	Oropharyngeal swab	<10	<10	<10	NA	NA	NA	NA	NA	0/3	NA	NA
IFN-ω (TRU/ ml)	Serum	**10**	**2100**	**2100**	<10	<10	**17**	**10**	<10	5/8 (10-2100)	0/16 (<10)	0/16 (<10)
	Oropharyngeal swab	<10	**17**	**17**	NA	NA	NA	NA	NA	2/3 (17)	NA	NA

Data are expressed as single value for each patient (Patient No 1-8) or as median (range) and percentage. NAB positive patients are in bold. [+] COVID-19 Severity Index [9] was indicated for each patient. There are four risk categories based on values of COVID-19 Severity Index (0-2=low; 3-5=moderate; 6-7=high; ≥8=critical). *Decadron (Sigma Aldrich, St. Louis, MO, USA) was injected at 6 mg per day for ~8 days. Veklury (Gilead Sciences, Foster City, CA, USA) was administered at 200 mg during the first dose, and at 100 mg in the following 4 days. Patients received a single administration of a monoclonal antibody-based combination therapy, which included a single infusion of Bamlanivimab and a double infusion of Etesevimab (Lilly, Indianapolis, IN, USA) at 700 mg/20 ml. All patients received low molecular weight heparin for prophylaxis of deep vein thrombosis as recommended at the time by the Italian Society of Infectious Diseases. **NAB detection was carried out at the time of hospitalization for patients No.1, 2, 3, 4, 7 and 8 or before starting Bamlanivimab-Etesevimab therapy for patients No.5 and 6 who were not hospitalized. ^Anti-IFN-α NAB were detected against IFN-α2 subtype and multiple IFN-α subtypes contained in the natural IFN-α preparation (IFN-αn1, Wellferon Glaxo Wellcome, Beckenham, United Kingdom). NAB titers were calculated using the Kawade's method, and the titers were expressed in Ten-fold Reduction Units (TRU)/ml. No NAB were detected in the serum of HIV-1 mono-infected individuals and healthy donors. Abbreviations: NA=not available.